Prostaglandin $F_{2\alpha}$ and GnRH Administration Improved Progesterone Status, Luteal Number, and Proportion of Ovular and Anovular Dairy Cows with Corpora Lutea Before a Timed Artificial Insemination Program

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Summary

The objective of this research was to increase the proportion of cows with at least 1 functional corpus luteum (**CL**) and elevated progesterone at the onset of the timed artificial insemination (**TAI**) program. Postpartum Holstein cows in 1 herd were stratified by lactation number at calving (September 2009 through August 2010) and assigned randomly to 1 of 2 treatments: (1) Presynch-10 (n = 105): two 25-mg injections of prostaglandin $F_{2\alpha}$ (**PG**) 14 days apart (Presynch); and (2) PG-3-G (n = 105): one 25-mg injection of PG 3 days before 100 µg gonadotropin-releasing hormone (**GnRH**; Pre-GnRH), with the PG injection administered at the same time as the second PG in the Presynch-10 treatment. Cows were enrolled in a TAI protocol (Ovsynch; injection of GnRH 7 days before [GnRH-1] and 56 hours after [GnRH-2] PG with AI 16 to 18 hours after GnRH-2) 10 days after the second or only PG injection. Blood samples for progesterone or estradiol analyses were collected on median days in milk (**DIM**): 36, 39, 50, 53 (Pre-GnRH), 60 (GnRH-1), 67 (PG), 69 (GnRH-2), and 70 (TAI). Ovarian structures were measured by ultrasonography on median DIM 53, 60, 67, 69, and 6 days post-TAI to determine follicle diameters, ovulation response to GnRH, or both. Although progesterone concentration did not differ between treatments before Pre-GnRH injection, the proportion of cows with at least 1 CL tended to be greater for PG-3-G than Presynch-10 cows, and more PG-3-G cows ovulated after Pre-GnRH than ovulated spontaneously in Presynch-10. Furthermore, diameter of follicles that ovulated tended to be smaller in PG-3-G than Presynch-10 cows after Pre-GnRH. At GnRH-1, the proportion of cows with progesterone ≥1 ng/mL, the number of CL per cow, and the proportion of cows with at least 1 CL were greater for PG-3-G than Presynch-10. Neither follicle diameter nor percentage of cows ovulating after GnRH-1 differed between treatments. At PG injection during the week of TAI, progesterone concentration and the proportion of cows with progesterone ≥ 1 ng/mL tended to be greater for PG-3-G than Presynch-10, and PG-3-G had more CL per cow than Presynch-10. No ovarian characteristics differed between treatments after GnRH-2, including progesterone concentration, number of CL per cow, and total luteal volume 7 days after GnRH-2.

Many of the previous ovarian traits were improved in both ovular and anovular cows after PG-3-G compared with Presynch-10. Pregnancies per AI at days 32 and 60 were only numerically greater for PG-3-G vs. Presynch-10 cows, largely because of differences detected during months without heat stress. We concluded that the PG-3-G treatment increased ovulation rate and luteal function 7 days before the onset of Ovsynch, resulting in improved follicular synchrony and predisposing potentially greater pregnancies per AI in lactating dairy cows.

Key words: luteal function, ovulation, presynchronization, progesterone, timed artificial insemination (TAI)

Introduction

Timed artificial insemination (**TAI**) programs facilitate control of reproductive cycles in lactating dairy cattle and provide viable options to AI programs solely based on detection of estrus. Preliminary studies formed the foundation for developing a TAI program when gonadotropin-releasing hormone (**GnRH)** administered to control follicle waves was followed in 6 or 7 days by prostaglandin $F_{2\alpha}$ (**PG**) to regress functional luteal tissue in a coordinated fashion. According to a survey of 103 Alta Genetics progeny-test herds, development of the Ovsynch protocol (injection of GnRH 7 days before [**GnRH-1**] and 48 hours after [**GnRH-2**] PG with TAI administered 16 hours after GnRH-2) led to its adoption in more than 85% of these large (> 500 cows per herd) U.S. dairy herds; nationally, the mean percentage of 231,288 cows inseminated after a TAI protocol was reported to be 43.4% and differed among 4 regions of the U.S.

Lactating dairy cows treated with the Ovsynch program beginning from days 5 through 12 of the estrous cycle had greater incidences of ovulation and pregnancies per AI (**P/AI**) than cows treated at other stages of the cycle. On the basis of the hypothesis that fertility after a TAI program was related to the stage of the estrous cycle (or stage of the first follicular wave), presynchronization of estrous cycles was attempted before the Ovsynch program by using 2 injections of PG administered 14 days apart (**Presynch**). The second Presynch injection given 12 d before the onset of the TAI program resulted in a larger proportion of cows in diestrus at the onset of the TAI program. These cows had greater P/AI than cows initiating the TAI programs at random stages of the estrous cycle, as did cows in subsequent experiments in which estrous cycles were presynchronized after administration of 1 or 2 presynchronizing injections of PG.

Therefore, the interval between the standard second Presynch PG injection and the onset of Ovsynch is important to the stage of estrous cycle or stage of follicular wave in which cows are found at the time of GnRH-1. Assuming that luteolysis occurs from 0 to 5 days after the second Presynch PG injection, intervals of 14 (Presynch-14), 12 (Presynch-12), 11 (Presynch-11), and 10 days (Presynch-10) would synchronize a majority of the cows to days 9 through 14, days 7 through 12, days 6 through 11, or days 5 through 10 of the cycle. On the basis of studies in which GnRH was given at various stages of the first follicular wave, intervals of 10 or 11 days (days 5 through 11 of the cycle) should facilitate greater ovulatory responses to GnRH-1. Moreover, Presynch-14 was concluded to decrease ovulatory responses to the first and second GnRH injections and to result in lesser P/AI compared with an 11-day interval.

The objectives of the present study were to test which of 2 presynchronization methods administered before a TAI program produced the greatest percentage of cows having a functional CL and elevated progesterone concentrations before enrollment in a TAI program to increase subsequent P/AI. The first treatment selected in which both $PGF_{2\alpha}$ and GnRH were administered before applying the Ovsynch protocol (Peters and Pursley, 2002) is similar to Double Ovsynch, but it excludes the initial GnRH injection of the presynchronization portion of Double Ovsynch. The second treatment represents what should be the best standard Presynch protocol in which the interval from the second Presynch $PGF_{2\alpha}$ injection to the onset of the Ovsynch protocol was reduced from 14 to 10 days. This treatment (Presynch-10) should result in more cows that are earlier in their estrous cycle and first follicular wave (days 5 to 10) at the onset of the Ovsynch protocol than a Presynch-14 program in which most cows would be later (days 9 to 14) in their estrous cycle at the first GnRH (GnRH-1) injection of the TAI program.

Experimental Procedures

Lactating Holstein cows were enrolled at calving from September 2009 through August 2010 at the Kansas State University Dairy Teaching and Research Center in Manhattan. Cows were housed in covered free stalls and fed twice or thrice (summer) daily a total mixed ration calculated to meet nutrient requirements for lactating dairy cows producing 110 lb of 3.5% milk (NRC, 2001). The diet consisted of alfalfa hay, corn silage, soybean meal, whole cottonseed, corn or milo grain, corn gluten feed, vitamins, and minerals. Cows were milked every 8 hours in a double 6 Herringbone milking parlor.

At calving, 210 cows enrolled in the study were stratified by lactation number (1 vs. 2+) and assigned randomly to receive 1 of 2 presynchronization treatments (Figure 1). The first treatment (**PG-3-G**) consisted of a 25-mg i.m. injection of PG (Pre-PG; 5 mL Lutalyse, Pfizer Animal Health, Madison, NJ) 3 days before a 100-μg i.m. injection of GnRH (Pre-GnRH; 2 mL Fertagyl, Merck Animal Health, Whitehouse Station, NJ). The second treatment (**Presynch-10**) was timed so the second of two 25-mg i.m. injections of PG (5 mL Lutalyse, Pfizer Animal Health) was administered on the same day as the Pre-PG injection in the PG-3-G treatment (Figure 1). The Ovsynch TAI program was initiated 10 days after either the Pre-PG or Presynch PG-2 injection. Treatment injections were staggered within cluster so all cows were inseminated on the same day every 2 weeks (Figure 1).

At calving, a new breeding cluster of cows was initiated every 2 weeks. Body condition scores (**BCS**; 1 = thin, 5 = fat) were assigned and pregnancies were diagnosed as illustrated in Figure 1. The monthly Dairy Herd Improvement test day energy-corrected milk (**ECM**) yield after 60 DIM near the onset of treatment was recorded for cows enrolled in the study. Three technicians performed inseminations, with 1 technician conducting more than 85%. Multiple sires were used. Pregnancy diagnosis was conducted by transrectal ultrasonography (5.0 MHz linear-array transducer, Aloka 500V, Corometrics Medical Systems, Inc., Wallingford, CT) on days 32 and 60 after TAI. A positive pregnancy outcome required presence of anechoic uterine fluid and a CL ≥25 mm in diameter or anechoic uterine fluid and presence of an embryo with a heartbeat.

Blood samples were collected by puncture of caudal vessels into evacuated tubes as indicated in Figure 1 to later assess concentrations of progesterone in blood serum. Ovarian scans were conducted by transrectal ultrasonography (Figure 1) to measure all ovarian follicles and determine when ovulation occurred after GnRH injections.

Results and Discussion

More ($P < 0.05$) PG-3-G cows ovulated after the Pre-GnRH injection than spontaneously ovulated in the Presynch-10 treatment (Table 1). As a result of this ovulatory response to the Pre-GnRH injection, a number of further differences were detected between treatments 7 days later when the Ovsynch protocol was initiated with the GnRH-1 injection (Table 1). More PG-3-G cows had at least 1 CL, more PG-3-G cows had elevated progesterone concentrations, and the average CL per cow was greater in the PG-3-G cows than in Presynch-10 cows (Table 1). The ovulation response to GnRH-1 did not differ between treatments, but more PG-3-G than Presynch-10 cows had multiple ovulations (Table 1).

Before the Ovsynch PG injection was administered, more PG-3-G than Presynch-10 cows had elevated progesterone concentrations, and average progesterone concentration tended to be greater (Table 1). In addition, the average number of CL per cows was greater for PG-3-G cows. No other differences were detected at the time of GnRH-2 or in response to GnRH-2. Incidences of CL regression after PG and percentages of cows ovulating after GnRH-2 did not differ between treatments.

In this preliminary study, pregnancies per AI were numerically greater for PG-3-G than Presynch-10 cows at days 32 and 60, particularly during the moderate to cold weather (October to May) months of the study. Fertility was very poor during the summer of 2010 (Figure 2), as evidenced by poor P/AI observed when the heat index exceeded 72.

Anovular cows, those which had not initiated estrous cycles before the treatments were applied, had numerous positive responses to the PG-3-G compared with the Presynch-10 treatment. These included greater ovulation response to the Pre-GnRH injection, more cows with at least 1 CL, greater progesterone concentration, and more CL per cow before the GnRH-1 injection of Ovsynch. The results indicate that anovular cows may be better candidates for the PG-3-G treatment even if fertility may not be improved. Furthermore, pregnancy results for both treatments during moderate to cold months were promising at days 32 and 60 of gestation.

Table 1. Selected outcomes after treatment

	Treatment[1]	
Item	PG-3-G	Presynch-10
Cows, no.	105	105
Ovulation after Pre-GnRH, %	80.0[a]	53.3[b]
GnRH-1		
Cows with corpora lutea (CL), no.	94.3[a]	76.2[b]
CL per cow, no.	1.2 ± 0.1[a]	0.9 ± 0.1[b]
Cows with progesterone ≥ 1 ng/mL, %	90.5[a]	76.2[b]
Ovulation, %	79.0[a.]	69.5[a]
Multiple ovulation, %	21.7[a]	8.2[b]
Ovsynch PG		
Cows with progesterone ≥ 1 ng/mL, %	93.3[c]	85.7[d]
Progesterone, ng/mL	5.9 ± 0.3[c]	5.0 ± 0.4[d]
CL per cow, no.	2.0 ± 0.1[a]	1.5 ± 0.1[b]
Pregnancies per artificial insemination (AI) (day 32), %	40.0[a]	33.3[a]
Moderate to cold	59.1	45.1
Summer	7.7	8.8
Pregnancies per AI (day 60), %	35.9[a]	30.5[a]
Moderate to cold	54.7	42.3
Summer	5.1	5.9
Pregnancy loss, %	7.5[a]	8.6[a]

[a-b] Treatments differ ($P \leq 0.05$).
[c-d] Treatments tend to differ ($P < 0.10$).
[1] See Figure 1 for treatment descriptions.

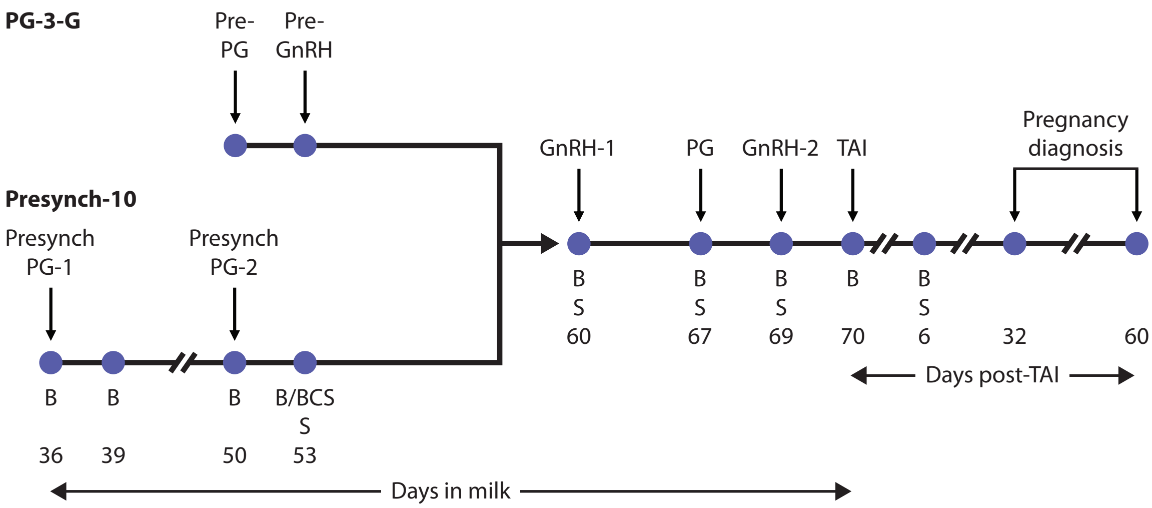

Figure 1. Experimental design of treatments and measurements.
At calving, lactating dairy cows were assigned randomly to 2 treatments, PG-3-G or Presynch-10. Cows received 100 µg GnRH at Pre-GnRH, GnRH-1, or GnRH-2; 25 mg of $PGF_{2\alpha}$ at Pre-PG, Presynch PG-1, Presynch PG-2, and PG. Blood (B) samples were collected by puncture of a caudal blood vessel and ovaries were scanned (S) by using transrectal ultrasonography. Positive pregnancy diagnoses required the presence of anechoic uterine fluid and a large corpus luteum or anechoic uterine fluid and presence of a viable embryo. BCS = body condition score; TAI = timed artificial insemination.

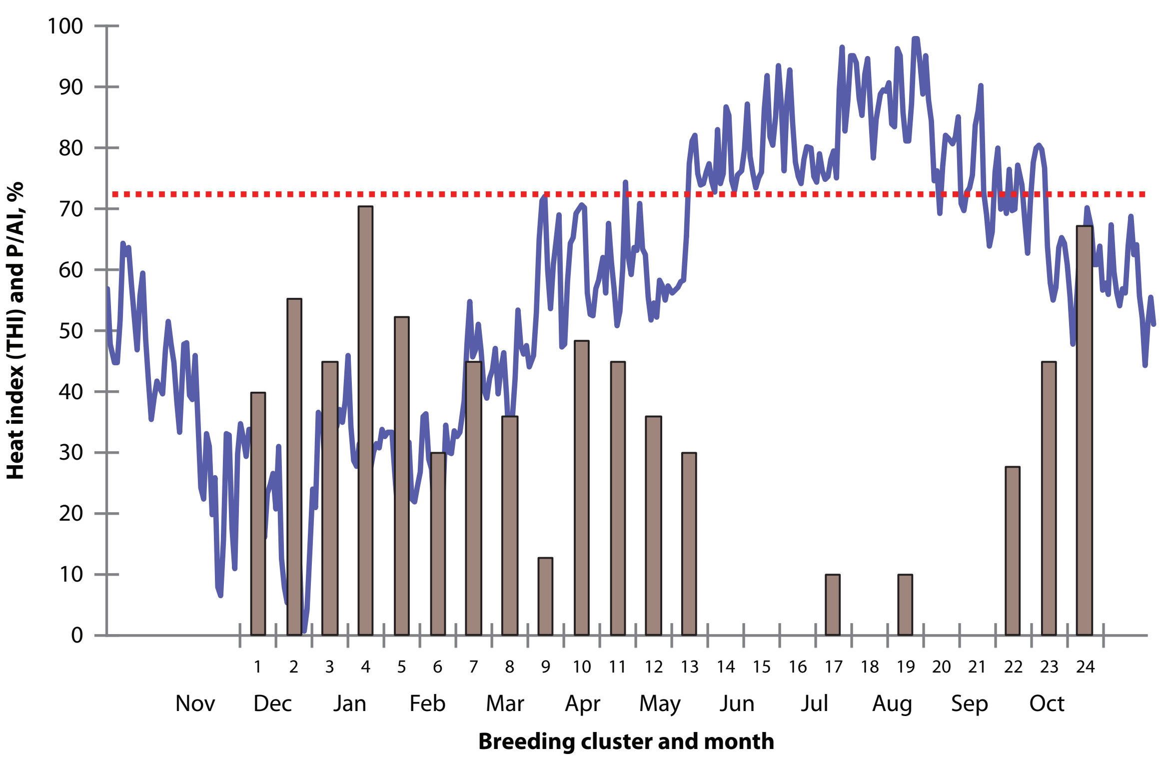

Figure 2. Pregnancies per artificial insemination (P/AI; bars).
P/AI assessed at day 32 post-timed AI in the 24 breeding clusters of cows inseminated during the experiment are superimposed over the average heat index (temperature-humidity index; continuous line) during the same period. The broken line (THI = 72) indicates where mild heat stress begins (mild = 72 to 79; moderate = 80 to 89; severe = 90 to 98; and danger > 98).